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PLUM WILT

Its Nature and Cause

By B. B. HIGGINS

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PLUM WILT

ITS NATURE AND CAUSES

(Numbers in the text refer to references on page 18).

A DISEASE of Japanese Plums and of hybrid varieties of Japanese parentage, known locally as "wilt," has been under observation at this Station for several years. It was mentioned by Starnes [18] in 1904 as a very serious obstacle to growing these plums; and a further description of the outward symptoms by the same author [19], followed the next year. He thought the disease was of bacterial nature similar to pear blight.

In 1910 after several inoculation experiments Stuckey and Temple [20] decided that the disease was of fungous rather than bacterial origin; but did not pursue the study further.

During the fall of 1913 the investigation herein reported was begun by the author to determine the identity of the causal organism, the means of infection and spread of the disease, its course of development, and the possibility of controlling the trouble.

SYMPTOMS

Very often the first noticeable indication of the unhealthy condition of the tree is the sudden wilting of the leaves on the whole tree or on a single branch. This is usually the case when the disease makes its appearance in spring or early summer. At the base of the wilting portion the bark is dead, the wood dark brown, or black, and the cambium is dead and blackened for some distance under the green bark. In case only a single branch is wilted the wood is found discolored around the heart usually throughout the trunk of the tree and the larger branches. Quite frequently also the bark and wood are dead along one side of the trunk to a wound near or below the surface of the soil, where infection has occurred. In many cases however no surface wound is found, the wound where the fungus gained entrance having been overgrown. The entire tree usually dies within a year from the wilting of the first branch.

One can sometimes locate diseased trees before wilting begins, by the scorched appearance of the leaves during dry weather in late summer. The wood in the center of such trees is often found to be diseased and partly filled with gum. The decreased rise of water due to partial plugging of the conducting tissue probably causes the edge of the leaves to dry. This appearance of the leaves is not a specific symptom of wilt however, as it may be caused by anything which curtails the supply of water to the leaves. It often occurs in trees with a diseased root system and frequently in perfectly healthy trees which are growing on hard dry soil.

In trees that die during early summer there is usually little surface gum flow; but late in the season diseased trees are often attacked by bark

beetles and gum flows freely from the openings. The beetles probably do not induce gum formation, but merely furnish an opening for passage to the surface of gum already formed. Such trees usually die during the fall and winter and fail to put forth leaves the following spring.

ISOLATIONS AND INOCULATIONS

During the fall and winter of 1913 several isolations were made from partly dead trees, by placing on nutrient agar small chips of wood removed with a flamed scalpel from the diseased area. In this way five different fungi were isolated and grown in pure culture. The parasitism of each was tested by inoculations on two year old trees growing in pots in the green house during the winter and early spring of 1914.

In the first series two trees were inoculated with each fungus to be tested; and each tree was inoculated at several (4-20) points usually near the base of a young twig. In about half the inoculations in each set the bark was slightly broken and a bit of fungous mycelium placed directly over the wound. In the other half the fungus was placed directly on the surface of the unbroken bark. All were then covered with moist absorbent cotton which was kept moist for several days by spraying twice daily. As checks, two trees were similarly wounded and wrapped with absorbent cotton but not inoculated. The checks and all the inoculated trees except one set remained healthy and the wounds healed within a very short time without any gum formation. In the one set inoculated with D No. 3* several of the wounded points showed gumming and one twig died five days after inoculation.

Two other trees were then inoculated at a total of fifteen points, by slitting the bark and placing a bit of the fungous (D No. 3) mycelium in the cut. The wounds were wrapped with absorbent cotton and kept moist as in the previous experiment. Two check trees of the same varieties were similarly wounded and wrapped but not inoculated. As in the previous test the check wounds healed normally without gum formation. In the inoculated trees all the wounds were gumming and three twigs were dead at the end of four days. The bark was killed for some distance (one to three centimeters) around each wound, and the cambium was blackened for some distance beneath the still living bark very much as in diseased trees in the orchard. The wood around the point of inoculation as well as the base of the wilted twigs was black and filled with gum. Chips of this blackened wood and cambium all gave pure cultures of the fungus D No. 3 when removed aseptically and placed on nutrient agar.

This set where the fungus was inserted into the wound showed so much higher infection than the first that a similar series was run with each of the isolated organisms. Checks were also similarly wounded and wrapped but not inoculated. Again none of the checks and only the set inoculated with D. No. 3 showed any gumming or sign of infection. In the set inoculated with D No. 3 all of the thirteen points were gumming by the seventh day and eleven twigs wilted. In most cases pycnidia of the fungus

* Indicating sources and isolation of the fungus.

developed in the dead bark around the points of inoculation in from three to five weeks.

In trying out cultures from various sources further inoculations were made in the green house as follows: On May 5, 1914, one Satsuma, one Red June, and one Abundance tree were wounded and inoculated at a total of 29 points, twenty-seven of the twenty-nine inoculations produced infection and two twigs died. The Abundance tree finally died during August, 1915. On May 15th one Satsuma and one Red June were inoculated at a total of 44 points. All produced lesions and gumming and eleven twigs wilted.

A five year old apricot tree was wounded and inoculated on one branch (four points) with D No. 3, on a second branch (five points) with *Valsa leucostoma*, and a third branch wounded (three points) but not inoculated neither the check wound, nor those inoculated with *Valsa leucostoma* showed any gumming or other signs of infection. Those inoculated with D No. 3 all produced considerable gum. The bark was killed around each wound and a one year old branch wilted.

Valsa leucostoma appears quite commonly on dead trees in the Station orchard, and although it was never isolated from the wood of wilting trees its parasitism was tested by inoculations with mycelium obtained both from ascospores and from conidia. In all, 44 inoculations on plums were made in the green house by slitting the bark and inserting bits of mycelium from pure cultures. Of these 44 inoculations slight gumming was produced by 14. The wounds were enlarged very slightly and in most cases gumming ceased and the wounds healed after a few weeks. Very similar results were also obtained on older trees in the field. It scarcely seems reasonable therefore to suppose that such a weak parasite could cause the death of vigorous trees with the symptoms produced by the wilt disease. From various observations and isolation tests this fungus appears to inhabit the bark of dying and dead trees and rarely enters the wood until some time after death of the trees. In orchards examined at Knoxville, Ga., where the wilt disease was very destructive, *Valsa* was not found at all.

Hundreds of isolations from wood of diseased trees show that the fungus D No. 3 is constantly associated with the wilt. In many cases pure cultures have been obtained directly from the small chips of diseased wood placed on nutrient agar. On April 17, 1914 a tree was noted one branch of which was already dead and another just wilting. One side of the trunk was dead to the ground. A block was cut from the base of the tree (Fig 13) near the juncture of sound and diseased tissue, and chips of the diseased tissue placed on nutrient agar in petri dishes. Many of the chips gave pure culture of this fungus. Four such chips with the fungous developed are shown in figure 23.

The same fungus was also isolated from a wilting branch of Bradshaw plum (*P. domestica*), from wilting plum trees (Funks Early, a Japanese bybrid) from Knoxville, Ga., from a wilting branch of *P. injucunda* Small, and from a sweet cherry (*P. avium*) tree which had just wilted.

Inoculations in the Field—To learn something of the course of the disease in mature trees, several inoculations were made in older trees growing outside.

On May 22, 1914, four plum trees (eight years from setting) three Japanese varieties, one Bartlett, and two Ogon and one European or P. domestica variety, Imperial Gage were inoculated at a total of eight points. The bark, where inoculation was to be made, was thoroughly scrubbed with absorbent cotton dipped in alcohol. A slit was then made with a sterile scalpel, a bit of the fungous mycelium inserted, and the wound covered with a thick sheet of grafting wax. Five days later gum formation had become so extensive that the wax covering was broken and gum protruded from each of the wounds. Large dead areas soon formed; but none of the trees died during the first year. During the summer and fall of 1915 the Imperial Gage and one of the Ogon trees died. The original fungus was isolated from the branches and roots of the trees, and pycnidia were formed on branches of the Imperial Gage. On the other Ogon tree some of the branches immediately above the inoculations developed practically no twig growth and will probably wilt next spring. The base of the Bartlett tree was photographed on August 23, 1914, (Fig. 22). This shows gum breaking through the bark at points from the wound up into one of the branches, indicating that the fungus is growing near the surface in this region.

On May 22nd further inoculations were made on five to six years old seedling trees which had grown up in an old trash dump, using the same method as used in the orchard. The trees inoculated included three seedling plums of Japanese type, three wild plums (P.angustifolia) two peach, and two wild cherry. One week later all the wounds were gumming. At the end of a month large cankers or dead areas had been formed and one branch on a wild plum and one on the wild cherry had wilted. The following spring one of the plum trees wilted (Fig. 21), just after blooming and before the leaves had developed. The fungus had not yet reached the roots and vigorous sprouts soon sprang up. Along one side and towards the center, however, the wood was discolored and filled with gum for eight inches below the point of inoculation, and isolations from the edge of this blackened area gave nearly pure cultures of D No. 3. On August 18, 1915, one of the inoculated peach trees was cut and examined. A dead area eighteen inches long and extending half way round the tree had been formed, but was partly overgrown during the past summer. The wood in this area was dark brown almost black and filled with gum contrasting sharply with the white normal wood. Cultures from the discolored wood showed that the fungus was still alive and vigorous.

On two sweet cherry trees seven inoculations were made on May 19, 1915, and the same number were made on Mahaleb cherry the same day. The bark was washed with alcohol and inoculated as in the last series. As checks in each case seven wounds were similarly made but not inoculated. Five days later all the inoculated wounds were gumming, those of the Mahaleb quite profusely, four small branches of the sweet cherry had wilted, and a few days later one of the Mahaleb branches died. The checks all healed normally.

EFFECT OF SEASON OF INFECTION

The fact that the spread of the fungus appears to be limited by gum formation in the wood was noticed very early in the investigation. It has been claimed by Butler [4] and others that gum formation occurs only

when growth is active. It was thought therefore that the season in which infection occurs might influence considerably the course of the disease. That is if infection should occur during the dormancy of the tree, the fungus might be expected to develop unobstructed and soon spread throughout the tree and cause wilting as soon as growth started in the tree. To test this supposition, several inoculations were made during the winter of 1914-15. On December 18th two trees were repotted, brought into the green house and inoculated at three points each. The bark was washed with alcohol, slit with a sterile scalpel and a bit of the fungous mycelium inserted. The wound was then wrapped with absorbent cotton and kept moist for the first three days. The temperature of the green house was kept rather low and no sign of growth activity of the trees was visible at the end of two weeks when the cotton was found to be stiff with gum which had exuded from the wounds. One branch died just as the buds began to open, and another died soon after. Other branches immediately above infection points were stunted and made very little growth until late in the season.

On January 6 two other trees were brought into the green house. Later, just as the buds began to swell they were inoculated in the same manner except that the wounds were wrapped with grafting wax instead of cotton. The results were very similar to the other. One branch died just as buds began to open and others were stunted in growth.

In both cases the fungus seemed to spread slightly further through the cambial region; so that the surface cankers were slightly larger than usual, indicating that a gum formation in this region is not quite so rapid as during active growth.

On the same day, January 8, several trees in the trash dump were inoculated by the same method as in the preceding series. The trees inoculated included five plum seedlings of the Japanese type, eight of *P. angustifolia*, three of *P. serotina*, and three of peach. No outward sign of infection could be seen in any case even after growth had started in the spring. When the wounds were examined the fungus had dried up and appeared to be dead in all but three wounds. In one of the peach and two of the *P. angustifolia* there was a slight gum formation beneath the wax and a slight blackening of the wood immediately beneath the wound. The fungus was isolated from this blackened area but in no case had it penetrated more than a few millimeters into the wood.

Just why active infection did not occur from these inoculations is rather difficult to explain. Whether the failure was due to lack of moisture or to the low temperature or to a combination of the two can not be stated positively at present; but observations with cultures indicate that low temperature was the principal factor.

RELATION BETWEEN HOST AND PARASITE

One of the very interesting phenomena noticed in the study of this disease is the sharp limitation of the spread of the fungus, especially when inoculated into young growing tissue. When infection is made just below a young twig the bark and wood is often killed up one side within a few days; but unless it becomes entirely girdled within a week it usually sur-

vives and if growth is rapid the dead tissue may be overgrown the first season.

When such partly dead twigs were sectioned, it was found that there was a layer of gum-filled tissue surrounding the infected area. The vascular ducts as well as the wood parenchyma cells are filled with gum. The gum forms a temporary mechanical barrier to the further spread of the fungus. From the appearance of the mycelium in the gum-filled region it seems very probable that penetration of the fresh gum is prevented by an enzyme which dissolves the cell walls of the fungous mycelium. In longitudinal sections of recently infected twigs many dead and partly destroyed mycelial threads are found. A similar destruction of the mycelium of *Coryneum Beijerinckii* is mentioned by Beijerinck and Rant [3] who attribute it to a cytolytic enzyme from the dead host cells.

This mechanical limiting of the fungous advance is illustrated by the small size of the cankers produced in young trees and by the greater spread of the fungus through the cambial tissue and through the older wood towards the heart of the tree as shown in figure 18. It also suggests a very plausible explanation for the much greater destruction caused by the disease in older than in very young trees. Although some gum is formed throughout the trunk of large trees eight to ten years old, it is not formed in sufficient quantities to prevent the comparatively rapid spread of the fungus.

The fungous mycelium is at first white but soon turns dark almost black, helping to produce the dark coloration of the wood. It spreads mostly through the vascular ducts, passing from one to another through the connecting pits. Comparatively thin and delicate branches are also sent through the medullary rays. These seem to function principally as feeders and are usually bent and wound around inside the ray cells. Similar branches were found by Evans [6] in citrus fruits infected with *Diplodia natalensis* and were described by him as haustoria. The branches in plum wood are more extensive and have functions other than haustorial as usually understood, apparently serving to infect new ducts.

The fungus does not seem to produce any wood destroying enzyme in appreciable amount. The mycelium always passes through a pit in passing from the ducts to ray cells and from one ray cell to its neighbor (Figs. 11 and 12). These pits are not visibly enlarged. After the fungus had grown six months on blocks of sterile peach wood no enlargement of the pits could be detected.

Cause of Gum Formation—The physiology of gum formation in various species of plants has been the subject of frequent discussions for many years, same authors maintaining that gummosis is due to an enzyme produced by the host protoplasm while others assert that it cannot be due to euzymatic activity. While no one has demonstrated positively that the phenomenon is of enzymatic origin, the greater weight of evidence certainly points in this direction.

After a long series of experiments in producing gum formation in various species of Prunus by wounding, by parasitic fungi, and by applying certain chemicals Beijerinck [2] concludes that it is a necrobiotic

phenomenon induced by a cytase within the protoplasm of the plant, which escapes and attacks the neighboring cell walls when the protoplast is killed by any means. Soraner [14-17] from very similar experiments, concludes that gum formation is due to excess of hydrolyzing over coagulating enzymes which prevents the deposit of synthetized pectin and other carbohydrates in growing tissue and also allows the cell walls to be attacked. This inequality he attributes to an accumulation of acids, eg. tannic and oxalic, which inhibit the action of coagulating enzymes [2].

While both theories are open to certain objections they appear to be the two most plausible explanations yet advanced. From personal observations and from analysis of the observations and experiments reported by others, it seems less open to objection to assume that a cytase (or enzyme of whatever nature) forming zymogen exists throughout the protoplasm of the plant, and breaks down forming the enzyme whenever acted upon by chemicals, organic poisons, etc. This theory is in accord with established knowledge of enzyme formation in many other plants and furnishes a very reasonable explanation for all gum formation. In very young twigs, where gum formation is sometimes induced by wounding alone, the wilting of tissue adjacent to the wound is probably sufficient to cause enzyme formation. Were the enzyme present in active condition all wounds should cause some gum formation whereas it is only in exceptional cases that it occurs from wounding alone.

The wilt fungus as well as many other parasitic organisms probably produces some toxic substance which reacting directly or indirectly on the zymogen causes enzyme formation. In the mature living wood gum formation is considerably in advance of the fungous mycelium and appears first in the vessels and neighboring cells which are connected with the vessels by pits. According to this theory the toxic substance is produced by the fungus and passes up with the ascending water and through the pits comes into contact with living protoplasts in the adjoining cells. These secrete the enzyme which cause gelatinization of the primary lamella.

The gum is forced out through the pits into the vessel and also into the adjacent wood cells and fibers. Early stages (See Fig 9) in such gum formation are very abundant in recently infected plum branches, and always the gum appears first in the vessels and adjacent cells.

The deposit of gum in the wood, while limiting the spread of the parasite also injures the host plant by reducing more or less the supply of water and nutrients to the leaves and extremities of the plants. When the fungus attack is spread sufficient to cause gum formation through an entire cross section of the trunk or branch that tree or branch wilts, and death results apparently from lack of water. If the entire cross section is not filled with gum at once the growth of the host may be stunted and slowed up for a short time until new conducting tissues are formed. This may occur, as in some cases under observation, within a few weeks; or it may never occur at all, if the tree is old.

The gum is only a temporary barrier to the spread of the fungus, however. The fungus gradually grows through the gum destroying it, and then infects a new area. That this fungus has the ability to dissolve the

gum is indicated by its behavior in infected young trees which often live some years after the primary infection occurs. The history of such reinfections and final death of tree can often be traced in the trunk of the tree. In one tree seven years from setting, in which two of five large branches had wilted, the primary infection had occurred at some sort of wound on the trunk four years before. A large canker was formed the first year, but was almost entirely overgrown. Following this some of the new growth became infected but by the end of the third year the wound was overgrown. During this time, however, the fungus had spread throughout the interior of the trunk and branches, and after another year had reached the cambium of some branches when of course they died.

The fungus had been found fruiting on gum masses exuded from inoculated wounds, showing that at least some constituents of the gum may be used in the growth of the fungus.

This was more strikingly shown in pure cultures on exuded gum. October 29th gum which had exuded in large masses from plum trees during the previous six weeks was collected and soaked in water over night to soften the surface as much as possible. It was then a stiff plastic mass. About ten to twelve c.c. of this gum was placed in each of six test tubes, and steamed twenty minutes on each of three successive days. No change could be seen in the consistency of the gum at this time. Three of these were inoculated with the fungus and the other three held as checks. Four similar tubes were prepared and held eight days without heating. In two some organism, apparently Pennicillium, developed but the other two remained apparently sterile. One of these last was inoculated and the other held as a check. These two as well as the first set were incubated at 30° C. The fungus made rapid growth for a few days and at the end of three weeks the gum in one of the inoculated tubes had broken down and formed a perfect liquid. In each of the other three inoculated tubes part of the gum had become liquefied; but growth of the fungus had apparently ceased and solid lumps of gum were still present in these after six weeks. No visible change had occurred in the sterile gum except that the surface had begun to dry and turn somewhat darker. At this time one of the check tubes was also inoculated and the gum was entirely liquefied within five days.

CULTURES

In cultures the growth characters of the fungus were remarkably similar on nearly all media tried including bean agar, steamed snap beans, steamed corn meal, steamed oat meal, Irish potato plugs, sweet potato plugs, and on steamed leaves and twigs of plum. On all a luxuriant growth of mycelium was formed in a few days, at first white or light grayish, it began about the fourth or fifth day to change to a dark gray or black. Within about ten to fifteen days small tufts one to three millimeters in diameter could be seen forming over the surface of the media, which are found on examination to be peculiar stromatic pycnidia covered with long hairs (See fig. 2). These were often abortive and contained no cavity or spores. In other cases they contained more or less irregular cavities filled with colorless one

celled spores and the long slender paraphysis similar to those found later in plum bark.

In sections these bodies often appear to have several spore bearing cavities, but it was difficult to determine whether there were several cavities or merely one very irregular one. However, from study of serial sections of similar structures in plum bark it seems more likely that they each contained only one very irregular cavity.

Occasionally dark colored, once septate, spores were found free in the cultures which led at first to the supposition that they were clamydospores, but it was found later that they were merely pycnospores which had been exuded, and had turned dark and became septate after exposure to air.

Apples inoculated with the fungus were decayed in from ten to fifteen days. The decay was very soft and unlike that of *Sphaeropsis malorum*. Quantities of liquid oozed from the decaying apples and collected in the bottom of the vessel. No fertile pycnidia were ever produced. In some cases small black sclerotial structures, apparently rudiments of pycnidia developed just under the cuticle, but no cavities or spores ever developed in these although kept under observation several months.

On ripe apples the behavior was at first very similar, but later when the pycnidia like structures formed tufts of hairs were protruded through the cuticle, and spores developed in some. After about two months some hairy pycnidia were also produced over the surface of the decayed fruits as described for other media.

On pears the development of the rot and of pycnidia was very similar to that of apples. More surface mycelial growth was produced, probably because of the weaker epidermis and larger and more numerous lenticels.

Temperature Relations—Although no thorough investigation of the thermal death points or the minimum, optimum, and maximum growth temperatures have been undertaken, certain observations on effects produced by various temperatures seem to be worth recording especially since the fungus seems to be confined to warmer sections of this country.

In germinating spores, in hanging drops of water, some cultures were placed in an incubator held at 30° C, while others were left on the laboratory table. During the night the temperature dropped to 8° C. in the room, but the room temperature was raised to 18° C. the next morning. After eighteen hours the spores in cultures at room temperature had just begun to germinate while those at 30° C. had developed long germ tubes (Figs. 5 and 6). The former were then placed outside where the temperature continued below ten degrees, and the germ tubes had not developed farther after twenty-four hours, while those in the incubator had grown and branched widely, forming a complete mat in the drop of water.

Twelve hanging drop cultures in tap water were then prepared, inoculated with spores from plum bark, and placed two in incubator at 30° C., and eight in another, held at a temperature ranging between 7° and 10° C. Agar was poured in four petri dishes, allowed to harden, and then inoculated by dragging a needle point covered with spores across the plate at intervals of about two centimeters. Two of these were placed in each of the incubators.

The spores germinated in all the cultures at the highest temperature after a few hours, and after forty-eight hours had covered the plate. In the low temperature incubator the spores had not begun to germinate at the end of the fourth day. On the sixth day the temperature was allowed to go to 12° C., and after a few hours germ tubes had begun to appear. The experiment was repeated and run for eight days without any germination, but the spores germinated when the temperature rose slightly on the ninth day, indicating that the minimum temperature for spore germination is near 10° C.

At the same time, the limits of colonies growing in plates on bean agar were outlined by marking on the bottom of the plates with ink. Two were placed in the high temperature incubator, two in the low temperature incubator and two directly on a block of ice in the ice box. At the high temperature the colonies increased in diametor at the rate of about two centimeters each day, at the low temperature no measurable growth had occurred after five days but growth did occur at a little above 10° C. In the ice box no growth had occurred at the end of five days. Under the microscope a great many cells especially the tips of the mycelium had burst and the contents exuded in a mass. When placed in a warm incubator growth occurred but the colonies were very ragged indicating that parts of the mycelium were dead. These results also throw light on the failure of January inoculations to produce infections in trees outside. The records for that month show that the maximum temperature for the month was only a few degrees above the indicated minimum growth temperature, and under the grafting wax, where the temperature varied less, probably no growth occurred at all.

Another observation of interest was as follows: Some pieces of bark containing pycnidia were being kept in a moist chamber in the incubator and were accidentally allowed to remain after the temperature was raised to 60° C. After twenty-four hours at this temperature several hanging drops and agar plates were inoculated with spores from the pycnidia. Both the hyaline, continuous spores and those that had become dark and septate were used in each culture. The dark colored septate spores germinated normally while of the hyaline spores not one germinated. Apparently some associated change made the dark colored spores more resistant to deleterious effects of high temperature.

CHARACTERS OF FUNGUS

The pycnidia are somewhat variable in appearance depending on the conditions under which they were formed. They are sub-epidermal, erumpent or merely cracking the epidermis, oval or globose to elliptical and beakless with more or less stromatic thick walls. The pycnidial cavity is large and usually many chambered. Where the epidermis of the host is thin and readily broken the pycnidia are approximately globose and scattered, but on old branches or where the epidermis is thick and tough they are flattened or angular and aggregated four or five to several in a group. When scattered or developed so that there is no pressure on the sides there is considerable hairy development laterally underneath the epidermis.

On old trees the pycnidia are not common or easily found. The scarcity here is probably due to two causes: first the difficulty of breaking through the extremely thick cuticle of this group of plums, and second the fact that growth of the fungus is from the heart wood outward and because of the deposit of gum around the cambium rarely reaches the bark.

Just below the surface of the soil where the bark is continually moist pycnidia are quite commonly produced on the surface of the bark. These were mostly grouped about lenticels or other breaks in the bark and were more or less roughened and hairy.

Pycnidia were also produced abundantly in one case directly on the surface of the wood of a Mahaleb Cherry in an inoculated wound covered with grafting wax. In this case the pycnidia were separate globose to oval (200-250x150-200 mu.) and covered with short (mostly 20-30 mu. long) stiff hairs.

In all cases the spores were hyaline, or opaque from the extremely granular character of the protoplasm, and one celled so long as they remained in the pycnidium, but turned dark and became once septate on continued exposure outside the pycnidium. Often under moist conditions spores were extruded from the pycnidia in white masses or long, white tendrils which, in a few days, began turning dark. This behavior was noted on inoculated trees in the greenhouse and also in bark containing pycnidia when placed in a moist chamber.

Intermixed with the spores were long, slender paraphysis, which seemed to gelatinize to some extent under moist conditions. In material killed while the spores were being extruded the paraphysis stained poorly and a large per cent of them seemed to disappear entirely during the killing and staining processes.

NOMENCLATURE OF FUNGUS

From the fact that dark colored spores were found always outside the pycnidia and amongst the hairy covering, it was at first thought that they were clamydospores, and the fungus was tentatively placed in the genus *Sphaeropsis*[9], but closer observation showed the mistake as to the formation of the dark colored two-celled spores, and this character in connection with the presence of paraphysis and the complex stromatic character of the pycnidia indicated the relation of this fungus to the genus *Lasiodiplodia.*

There has been considerable discussion of recent years as to the nomenclature in the *Diplodia* group to which this genus belongs. Most of this discussion has centered around the systematic position of *Lasiodiplodia theobrome* (Griffin & Maublane), which has been placed in all of the four genera *Macrophoma*,[12] *Diplodia*,[8] *Botriodiplodia*,[11] and *Lasiodiplodia*[7]. Seeing the confusion that has arisen in separating the genera of the group Bancroft,[1] Van Hall and Drost,[22] and Taubenhaus[21] have in turn suggested lines of revision for the group. The two latter favor discarding the genus *Lasiodiplodia* and placing the species in the genus *Diplodia*, while Bancroft suggesting the need of revision, prefers retention of the genus *Lasiodiplodia* because of the presence of paraphysis.

Taubenhaus, after comparing the growth of *L. theobromæ*, *L. tubericola*, *Diplodia gossypina*, and *D. natalensis* on sweet potatoes in a moist chamber, comes to the conclusion that these species are congeneric, and also on the basis of this study concludes that the genera *Diplodiella*, *Chaetodiplodia*, *Lasiodiplodia and Batriodiplodia* "are not tenable," and should be combined with the genus *Diplodia*. He discards *Lasiodiplodia*, for instance, because the *Diplodia* species under consideration, *D. gossypina* and *D. natalensis*, produced hairy pycnidia, and the pycnidia of *D. gossypina* (*D. natalensis* not producing fertile pycnidia on sweet potato) contained paraphysis on sweet potatoes in a moist chamber. He was unfortunate in selecting for this comparative study two species of Diplodia which are almost typical of the genus *Lasiodiplodia*, as both might very properly be placed in the latter genus. Both normally have the pycnidia more or less grouped, and the pycnidia are paraphysite. And according to the illustration by Earle and Rogers [5] of the fungus which they consider *D. natalensis* the pycnidia are hairy, as in the original characterization of the genus *Lasiodiplodia*.

Taubenhaus states, however, that paraphysis were sometimes absent from the pycnidia of *D. gossypina*. This may have been brought about by the action of the killing and staining process as mentioned above, as occurring in material of the plum wilt organism under consideration. At any rate no non-paraphysate pycnidia have been found in any material of *D. gossypina* on cotton bolls collected either at this station or at Thomasville, Ga.

In the plum wilt fungus paraphysis were present in all live sporulating pycnidia examined. Some hollow cavities of dead pycnidia were found which contained no paraphysis; but neither did they contain sporophores. In fact the presence of paraphysis seems to be the most constant character of the pycnidia.

With these considerations it would seem to be unwise to discard *Lasiodiplodia* or other genera of the group until a thorough study of the characters can be made. That some of the genera might be combined is indidicated by the fact that of the species described under *Chaetodiplodia* in Saccardo, a very large per cent are described as having paraphysite pycnidia more or less aggregated, which would apparently show relation of at least these species to the genus *Lasiodiplodia*.

Until some such study can be undertaken it seems best to place the plum wilt fungus in the genus *Lasiodiplodia*. It is apparently very closely related to *L. Theobromæ* and also to *Diplodia natalensis;* but there are slight morphological differences in both cases. A comparative study may show it to be identical with either or both of these; but careful study of morphological and physiological characters in nature and on cultures media will be necessary to determine this question, or better still, comparison of perfect stages.

That cross inoculations can not be depended upon to determine relationship among such general parasites is indicated by the following results:

Early in the study of this disease a resemblance was noted between this fungus and *D. longispora* as well as the diseases produced (as des-

cribed by Miss Ingram [10]). For comparisons dried specimens of *D. longispora* on *Quercus prinus* were obtained from Dr. Haven Metcalf. There is only slight difference in the size, shape and development of the spores, but the pycnidia in *D. longispora* are more aggregated, not chambered, and non-paraphysite.

Later a fresh specimen of a chestnut twig inoculated with what is doubtless the same fungus from *Quercus prinus* was obtained from Dr. W. H. Rankin. From this specimen pure cultures of the fungus were obtained. On April 21, 1915, with this culture one Red June Plum (two years old in green house) was inoculated at thirteen points as in inoculations with the plum wilt fungus. Four days later all wounds were gumming, and four twigs immediately above points of inoculation were dead and two weeks later three more twigs had died.

On May 12th two other trees were inoculated in the same manner—one Red June at six points and one Abundance at nine points. Five days later all wounds were gumming and one twig on the Red June was dead.

On April 27th fourteen inoculations were made with the plum wilt fungus on species of oak. Twigs two to five years old were selected—four on *Q. rubra*, five on *Q. Alba*, and five on *Q. stellata*. The point to be inoculated was scrubbed with absorbent cotton dipped in alcohol. The bark was then slit with a sterile scalpel and a bit of mycelium inserted in each. As checks similar twigs were washed and wounded in the same way, but not inoculated. Six days later one of the twigs on *Quercus stellata* had wilted, and pure cultures of D No. 3 were obtained three inches from points of inoculation. Two weeks later another twig on *Q. stellata* wilted. The bark around wounds on the other species was killed and pycnidia were beginning to appear, but none wilted. All checks healed normally.

To compare more closely the action of the two fungi another series of inoculations on oak was made May 13th, as follows: On *Q. coccinea* with "Plum wilt" fungus five inoculations, with *Diplodia longispora* five inoculations, and checks six; and on *Q. stellata* with "Plum wilt" fungus five inoculations, with *D. longispora* nine inoculations, and checks eight. The inoculations were made in the same way as the previous series, except the wounds were covered with grafting wax instead of absorbent cotton, to prevent drying and entrance of other organisms. At end of two weeks two twigs on branches of *Q. stellata*, inoculated with D. No. 3, and all branches inoculated with *D. Longispora*, had wilted. None of those of *Q. coccinea*, inoculated with D No. 3, wilted. Those inoculated with *D. longispora* were either dead or dying; but were removed and burned at this time to prevent spread of the fungus as pycnidia were beginning to form.

Thus we see that while *D. longispora* is more active and destructive in oak than D No. 3, and the latter appears to be more active in plums, they are both capable of infecting the host normally attacked by the other, and were the morphological resemblance closer would usually be classed as identical.

In consideration of the difficulty of at present identifying this fungus with any of the heretofore described species of *Lasiodiplodia* or *Diplodia*,

it seems best to describe it as a new species. Since it does not seem that we are justified with our present knowledge of the group, in discarding the genus *Lasiodiplodia*, the fungus is placed in this genus and the name *Lasiodiplodia triflorae n. sp.* is proposed with the following description appended:

Lasiodiplodia triflorae n. sp. Pycnidia embedded in the cortex, subepidermal or sometimes on the surface of the bark, scattered or aggregated; ostiole not papillate; walls more or less thickened and stromatic, naked or variously covered with hairs; spores oblong, 22-25x13-16.5 mu. at first hyaline and continuous, but becoming dark brown and two celled after extrusion from the pycnidia, intermixed with paraphysis.

hab. trunk and branches of various species of *Prunus*.

Latin diagnosis: Pycnidiis sparsis vel gregariis, globosis vel ellipsoideis, non papillatis, subepidermicis aut raro epiphillis, non vel vix erumpentibus, sub-stromatibus, plus minusve comosis, intus uni.-v. pauciloculariibus; sporulis oblongis, 22-25x13-16.5 mu. diu granulato-hyalinis, et continuis, emersis et deum 1 septatis, fuscis; paraphysibus filiformibus, comixtis.

hab. truncis et ramnis *Prunii* sp.

ECONOMIC CONSIDERATIONS

The results of several inoculations show that the fungus can not gain entrance through the uninjured bark of the tree; but enters wounded trees and branches of any age, provided there is sufficient moisture to promote growth of the fungus.

Natural Infections.—Several cases of infection through wounds made in cultivation and pruning have been observed both on trunks and on branches. A typical case of this kind is shown in Figure 20, in which case the pruner had started to cut the branch but had left it after breaking the bark on either side. The branch wilted early in the following summer, and the causal organism was isolated from near the wound. Where a large branch is thus infected the fungus can readily grow down through the older wood in the center of the tree, reaching the trunk and other branches and finally causing the death of the whole tree.

Observations on both living and dead trees indicate that in a large majority of cases infection occurs on the trunk near the surface of the soil. Such trunk infections may occur through wounds made in cultivation, through open cankers initiated by *Bacterium pruni*, and through wounds made by the peach tree borer. The last two seem to be the most prevalent means of infection and may account for the greater mortality among trees of Japanese and Japanese hybrid varieties. These are much more susceptible to bacterial canker than either European or American varieties. Of the wilted trees removed from the Station orchard during the winter of 1914 fully one-third showed evidence of infection through wounds made by peach borers. In most of the others the original wounds were overgrown and it was impossible to determine their origin; but probably many of these had become infected through borer attacks when young.

The apparent preference of the borers for Japanese varieties is prob-

ably due to the peach stock on which these varieties are budded and in which the borers make their attack.

Under ordinary conditions there seems to be no very good reason from the grower's viewpoint for using peach stock. When nurserymen find some stock free from attacks of borers and otherwise satisfactory for this group we shall probably have gained much toward control of a disease which practically prohibits plum growing as a commercial venture in this section. Experiments to test other stocks for disease resistance are now under way, but several years must elapse before definite results can be obtained.

In the meantime the only means for controlling the disease pertains to preventing infection through wounds of all kinds. Borer runs should be cleaned out, the surrounding infected tissue removed, the surface of exposed tissue then washed with corrosive sublimate solution (1 part to 1000 of water), and then covered with grafting wax to prevent subsequent infection. Bacterial cankers and other wounds on the trunk and larger branches should be treated in the same way. When infection occurs in a branch it should be removed, as soon as noticed, including all discolored wood. Covering with grafting wax seems to hasten healing by preventing drying and death of adjacent tissues, but where many trees are to be treated it may be more economical to paint with white lead or coal tar preparations.

Rolfs [13] has found that bacterial canker can be controlled very thoroughly by removing cankers and spraying. The fact that these cankers may admit infection by the wilt fungus emphasizes the importance of using every means possible for control of bacterial canker.

SUMMARY AND CONCLUSIONS

1. Plum wilt has proven to be a very destructive disease, especially of Japanese and hybrid varieties of plums throughout this section. Reports of severe losses with symptoms pointing decidedly to this disease have come from eighteen localities in Georgia, also from North Carolina and from Alabama.

2. It is caused by a fungus, a new species of *Lasiodiplodia*, which infests principally the conducting tissue and the medullary rays of the wood, causing gum formations.

3. The sudden wilting is apparently due to loss of water supply from deposits of gum in the conducting elements of the wood.

4. The fungus cannot enter through the unbroken bark, but enters readily through wounds.

5. Observations show that a large per cent of infections occur through wounds produced by peach borers.

6. Suggestions are given for preventing infection through such wounds.

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EXPLANATION OF PLATES

Fig. 1. Section of pycnidium of **Lasiodiplodia triflorae n. sp.** in bark of plum twig.

Fig. 2. Free-hand section of pycnidium of same from culture, outlined only with camera-lucida.

Fig. 3. Portion of hymenial layer showing paraphysis and immature pycnospores.

Fig. 4. Mature but hyaline and one celled pycnospores.

Fig. 5. Same germinating in tap water after 18 hours at room temperature.

Fig. 6. Same germinating in tap water after 18 hours at 30° C.

Fig. 7. Pycnospores having become fuscus and two celled after exposure to air.

Fig. 8. Same germinating in tap water after 18 hours at 30° C.

Fig. 9. Cross section of duct and surrounding parenchyma cells, showing liquefaction of primary lamella and gum being pushed through the pits.

Fig. 10. Longitudinal section of young plum wood infected with **L. triflorae** showing gum and also some partly destroyed fungous mycelium in ducts.

Fig. 11. Longitudinal section of old wood showing fungous mycelium in duct, sending slender branches into medullary ray cells.

Fig. 12. Tangential section of sterilized peach wood on which **L. triflorae** had been growing six months, showing slender branches in medullary ray cells.

Fig. 13. Plum tree partly wilted. One branch wilted just as leaves were forming, and the second a few days before photographed on 4-17-14. Cut at base shows where block of diseased wood has been removed for study.

Fig. 14. Tree dead, last branch just wilting 6-4-14. Infected three years previously through wound on base.

Fig. 15. Branch with pycnidia of **Lasiodiplodia** showing as small swellings under the epidermis, nat. size.

Fig. 16. Tree showing poor growth because of gum formation in nearly all conducting tissue.

Fig. 17. Base of tree shown in Fig. 16, showing point of infection on left and small amount of white, healthy wood on right.

Fig. 18. Young tree one year after inoculation with **Lasiodiplodia** in the green house, with one side removed to show spread of infection through older central wood.

Fig. 19. Young tree inoculated with **Lasiodiplodia** in the green house, showing wilted twigs.

Fig. 20. Branch which has just wilted due to infection through wound made with pruning shears.

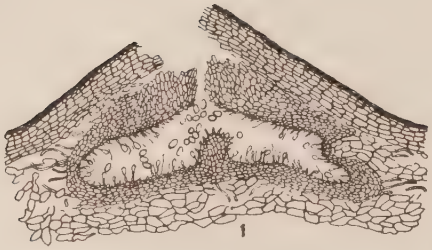
Fig. 21. Tree wilted while in bloom—inoculated during previous May—base of tree still living.

Fig. 22. Base of Bartlett plum tree 15 months after inoculation with **Lasiodiplodia**, gum exuding along one side of trunk and along one branch.

Fig. 23. Pure culture of **L. triflorae** obtained from base of tree shown in **Fig. 13.**

Fig. 24. Cross section of diseased twig showing limiting area of gum filled tissue and new sound wood at right.

Fig. 25. Longitudinal section of same.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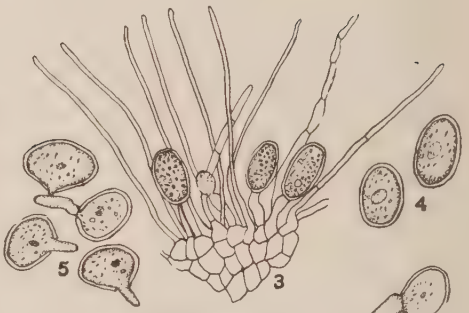
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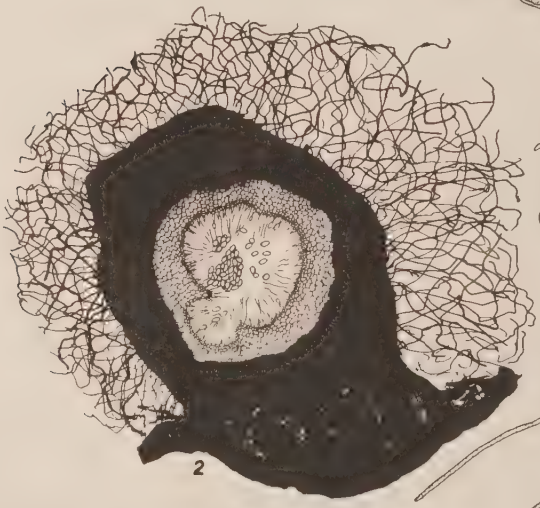
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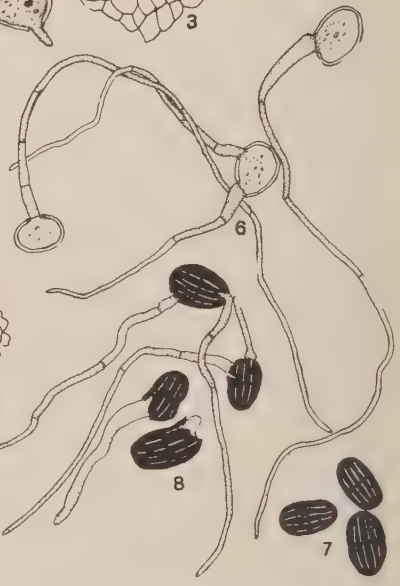
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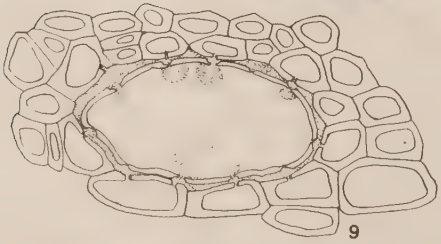
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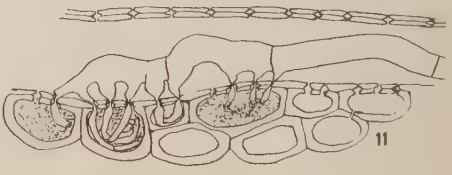
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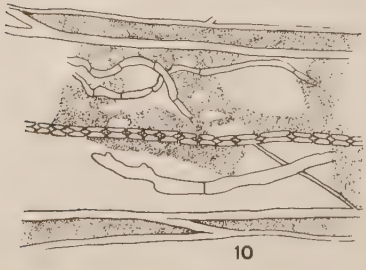
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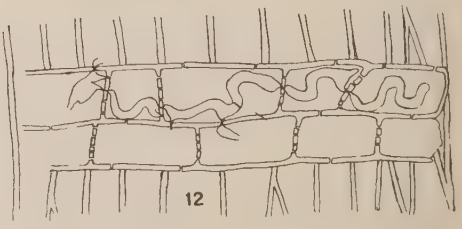
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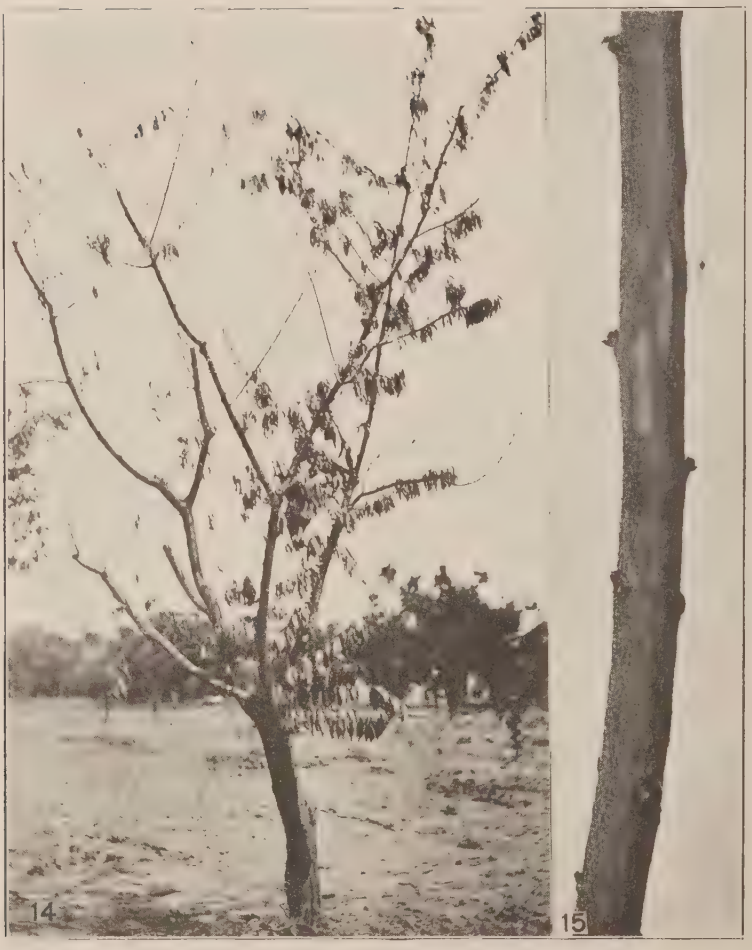


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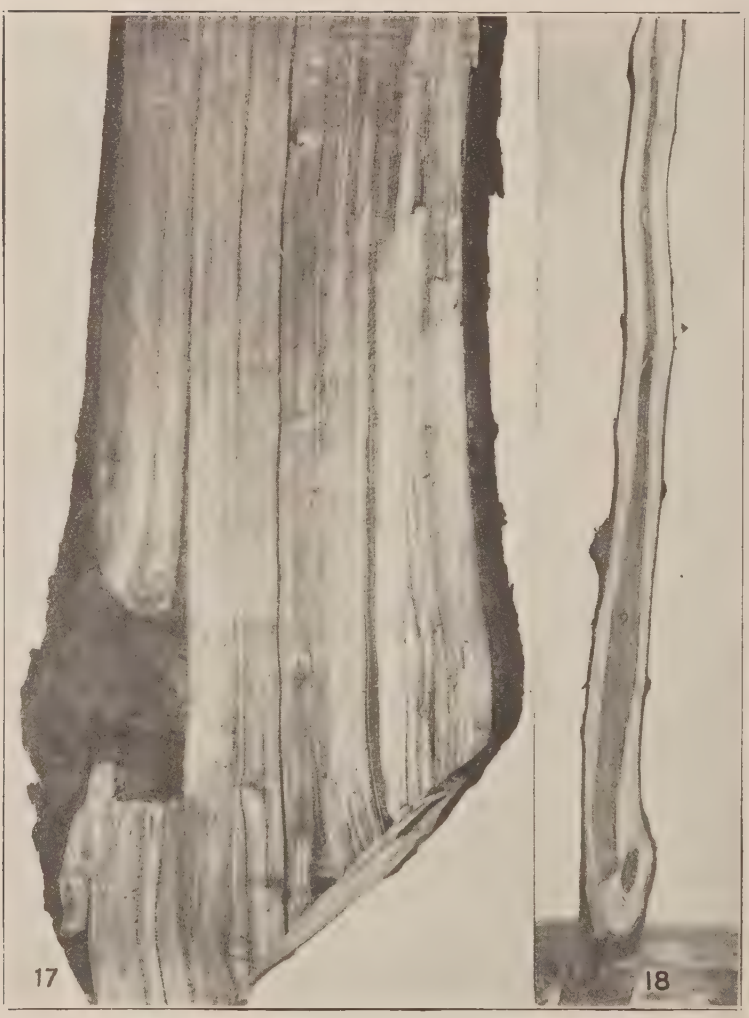
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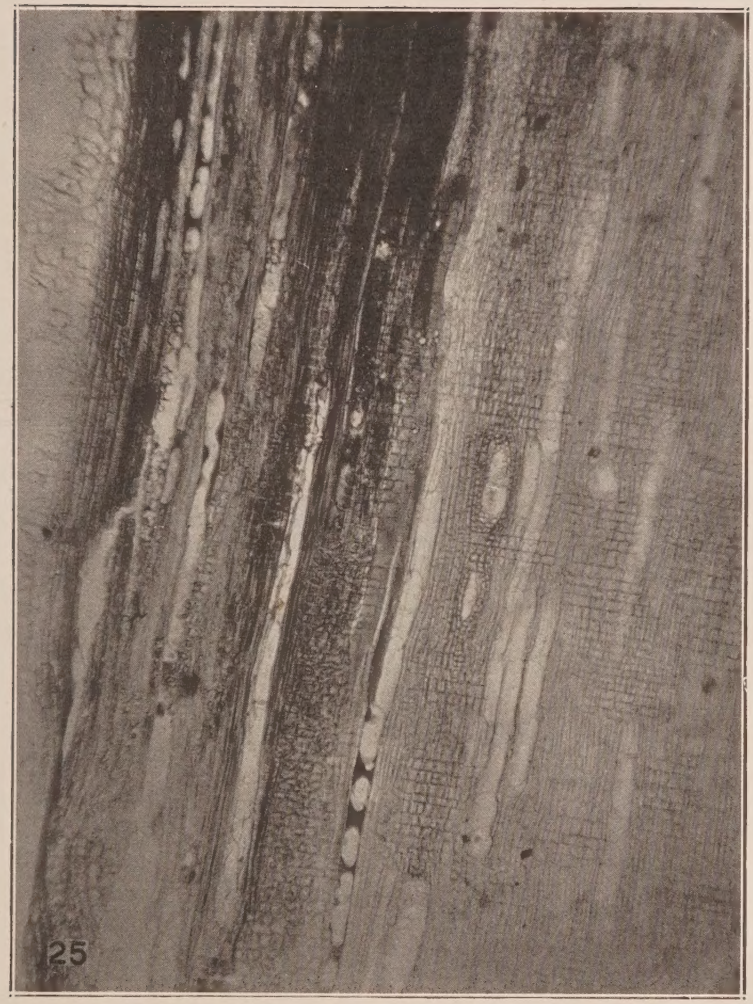
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